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and separating caveolae that are bound to the antibody from other materials in the initial fractions,

thereby producing purified caveolae.

REMARKS

Claims 1-9, 11-17, 19-22, 24-25 and 27-30 are pending. Claims 27-30 have been withdrawn from consideration.

Claims 1, 11, 19 and 24 have been amended to specify that the immunoisolation method comprises incubating the initial fractions with a monoclonal antibody that is specific for caveolin and which binds to oligomerized caveolin in its native state as an oligomeric structural cage surrounding intact caveolae. Support for this Amendment is found throughout the Specification, for example, at p. 5, lines 2-4; p. 7, line 29 through p. 8, line 2; and p. 13, line 11, through p. 14, line 14. No new matter has been added.

The remainder of the Remarks is set forth under appropriate headings for the convenience of the Examiner. The Remarks address issues raised in the Office Action, made final, in the parent application for the current RCE. The discussion is presented under appropriate headings in the order in which the issues were raised in that Office Action.

Summary of Invention

The invention is drawn to methods of producing purified caveolae, the methods including an immunoisolation step of incubating a sample containing plasma membranes with an antibody that is specific for caveolin and which binds to oligomerized caveolin in its native state as an oligomeric structural cage surrounding intact caveolae. The methods are simple and efficient means of producing purified caveolae which closely resemble caveolae in their native state (e.g., caveolae covered with the oligomeric structural cage of caveolin); the methods also minimize contamination and loss of molecules that dissociate from caveolae over time. Furthermore, the methods do not require perfusion of a tissue or coating of the plasma membranes with colloidal silica (described, for example, in US Patent 5,776,770), and thus allow a high level of flexibility of starting materials, as the methods can be used even for tissues or samples that cannot be perfused or coated with colloidal silica.

Rejection of Claims under 35 U.S.C. §102(b)

Claims 1-7, 11, 13-15 and 19-20 have been rejected under 35 U.S.C. §102(b), because the Examiner contends that they are anticipated by Stan *et al.* for the reasons set forth in the Office Action dated July 3, 2000. That Office Action states that Stan *et al.* describe subjecting fractions of plasma membranes to immunoisolation by polyclonal antibodies which "would inherently bind the oligomerized form of caveolae since they recognized the caveolae in its natural state, prior to being denatured."

In order for a reference to anticipate claims, the reference must teach every aspect of the claimed invention either explicitly or impliedly (see M.P.E.P. § 2131).

Stan *et al.* describe a method of purification of caveolae which includes immunoisolation of caveolae on anti-caveolin coated magnetic beads. However, the antibody used in the immunoisolation of Stan *et al.* differs significantly from the antibody described in the claimed methods. The Declaration under 37 C.F.R. §1.131 of Jan. E. Schnitzer, M.D. (herein referred to as the "Declaration,") describes in detail the differences between the antibody used in the Stan *et al.* reference and the antibody described in the claimed methods, and explains that one of ordinary skill in the art would not find the antibody used by Stan *et al.* to have the characteristics of an antibody as set forth in the claimed invention.

Briefly, Stan *et al.* describe a method of purification of caveolae which includes immunoisolation of caveolae on anti-caveolin coated magnetic beads using an antibody they had prepared themselves. This antibody, described as "anticaveolin-N" antibody, was separated from polyclonal sera raised in rabbits against synthetic peptides, which were not identical to any peptide found in caveolin, covalently coupled to keyhole limpet hemocyanin. Anticaveolin-N antibody was thus a fraction of a polyclonal antiserum, and not a monoclonal antibody as is set forth in the claims of the application.

Furthermore, one of ordinary skill in the art, recognizing that the synthetic peptide is 11/16 (less than 70%) identical to the N-terminal residues found in caveolin, would expect that the anticaveolin-N antibody fraction of Stan *et al.*, would likely have poor affinity to caveolin itself, as indicated not only by the overnight incubation time used by Stan *et al.* for isolation of caveolae, but also by the validation experiments shown in Figure 1 of Stan *et al.*. In contrast, the representative antibody used in the invention (CAV) is specific for caveolin, as shown by the ability of the representative antibody CAV to bind to the alpha-isoform of caveolin-1 via a specific epitope found in the N-terminal segment. Furthermore, the representative antibody CAV

also bound extremely rapidly (e.g., within one hour) and with high affinity to oligomerized caveolin in its native state as an oligomeric structural cage surrounding intact caveolae, as demonstrated in the application (see Example 2 and Figure 2, which depicts the rapidity of CAV binding in contrast with that of other antibodies including the rabbit polyclonal antiserum pAb).

The ability of the antibody used in the methods of the invention to bind to oligomerized caveolin in its native state as an oligomeric structural cage surrounding intact caveolae, as described in the application at page 13, line 11, through page 14, line 14, is reiterated by Oh and Schnitzer (Oh, P. and Schnitzer, J.E., *Journal of Biological Chemistry* 274(33):23144-23154 (1999), a copy of which is attached as Appendix I to this Amendment for the convenience of the Examiner). The Stan *et al.* anticaveolin-N antibody fraction does not appear to have the same ability to bind to oligomerized caveolin in its native state as an oligomeric structural cage surrounding intact caveolae: Stan *et al.* indicate that the anticaveolin-N antibody fraction was used for immunoisolation, and a commercial polyclonal antibody (pAb) was used for monitoring different experimental samples by Western blotting, thus suggesting the possibility that, at most, the anticaveolin-N antibody fraction and the commercial polyclonal antibody had comparable binding abilities for caveolin. As indicated by Oh and Schnitzer, the polyclonal antiserum pAb does not bind to oligomerized caveolin in its native state as an oligomeric structural cage surrounding intact caveolae (see Oh and Schnitzer, *supra*). Therefore, one of ordinary skill in the art would reasonably believe that the anticaveolin-N antibody fraction similarly would not bind to oligomerized caveolin in its native state as an oligomeric structural cage surrounding intact caveolae.

In view of these considerations, it is clear that Stan *et al.* do not describe a monoclonal antibody that is specific for caveolin and which binds to oligomerized caveolin in its native state as an oligomeric structural cage surrounding intact caveolae, as is used in the claimed methods of the invention. Thus, Stan *et al.* does not teach every aspect of the claimed invention either explicitly or impliedly, and the claimed invention is therefore not anticipated by the teachings of Stan *et al.*

Rejection of Claims under 35 U.S.C. §103

The Examiner rejected Claim 12 as being obvious over Stan *et al.*, stating that both shearing and sonication are recognized by those in the art to be used in disrupting membranes for caveolin purification.

Stan *et al.* describe the use of sonication as a membrane disruption method (page 597, first column, under "Purification of PVs" "Step III (Sonication)"). There is no teaching or suggestion in Stan *et al.* that any other membrane disruption method would produce different results upon immunoisolation of caveolae.

Applicants compared the fractions produced by sonication and by shearing (see the Specification in Examples 3 and 4, page 14, line 15, through page 16, line 19). It was determined that the fraction produced by sonication (the PC fraction) differed from the fraction produced by shearing (the V fraction). For example, the protein profiles indicated that sonication appeared to release additional low density vesicles into the PC fraction, that were not found in the V fraction (page 15, lines 4-10). Furthermore, the caveolin in the shearing fraction (V) was nearly all accessible and able to interact with immuno-beads during immunoisolation, resulting in a quantitative isolation of nearly all of the starting material. In contrast, when the sonication fraction (PC) was used, a significant amount of the caveolin was inaccessible to immunoisolation (page 16, lines 5-9). Use of shearing therefore allowed isolation of more caveolae than would be obtained when using sonication. Thus, Applicants have for the first time demonstrated a specific benefit to use of shearing, rather than sonication, as a membrane disruption method prior to immunoisolation.

Furthermore, even assuming *arguendo* that sonication and shearing were considered equivalent methods of membrane disruption, the claimed invention would nevertheless not have been rendered obvious by the teachings of Stan *et al.* As discussed in detail above, Stan *et al.* does not teach use of a monoclonal antibody that is specific for caveolin and which binds to oligomerized caveolin in its native state as an oligomeric structural cage surrounding intact caveolae, nor do they suggest use of such an antibody. One of ordinary skill in the art, given the teachings of Stan *et al.*, would not have been motivated to practice the methods of the invention which utilize monoclonal antibody that is specific for caveolin and which binds to oligomerized caveolin in its native state as an oligomeric structural cage surrounding intact caveolae. In view of these considerations, the claimed invention is not obvious over the teachings of Stan *et al.*

CONCLUSION

In view of the discussion presented above, the claims are in condition for allowance. Applicants respectfully request that the Examiner reconsider and withdraw all rejections.

If the Examiner believes that a telephone conversation would expedite prosecution, the Examiner is invited to contact Elizabeth W. Mata at (915) 845-3558. If Elizabeth W. Mata cannot be reached, the Examiner is invited to contact Doreen Hogle at (781) 861-6240.

Respectfully submitted,

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MARKED UP VERSION OF AMENDMENTS

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

1. (Amended) A method of producing purified caveolae, comprising the step of subjecting a sample of interest comprising plasma membranes to an immunoisolation method to separate caveolae from other materials in the sample of interest, wherein the immunoisolation method comprises incubating the sample of interest with [an] a monoclonal antibody that is specific for caveolin and which binds to oligomerized caveolin in its native state as an oligomeric structural cage surrounding intact caveolae, and separating caveolae that are bound to the antibody from other materials in the sample of interest, thereby producing purified caveolae.

11. (Amended) A method of producing purified caveolae, comprising the steps of:

providing a sample of interest comprising plasma membranes;

e) subjecting the sample of interest to a membrane disruption method, thereby producing a disrupted plasma membrane sample;

f) subjecting the disrupted plasma membrane sample to an immunoisolation method to separate caveolae from other materials in the disrupted plasma membrane sample, wherein the immunoisolation method comprises incubating the initial fractions with [an] a monoclonal antibody that is specific for caveolin and which binds to oligomerized caveolin in its native state as an oligomeric structural cage surrounding intact caveolae, and separating caveolae that are bound to the antibody from other materials in the disrupted plasma membrane sample,

thereby producing purified caveolae.

19. (Amended) A method of producing purified caveolae, comprising the steps of:

a) providing a sample of interest comprising plasma membranes;

b) subjecting the sample of interest to a membrane disruption method, thereby producing a disrupted plasma membrane sample;

c) subjecting the disrupted plasma membrane sample to a separation method based on density, thereby producing fractions of the disrupted plasma membrane sample, and collecting initial fractions of the disrupted plasma membrane sample;

d) subjecting the initial fractions of the disrupted plasma membrane sample to an

immunoisolation method to separate caveolae from the initial fractions, wherein the immunoisolation method comprises incubating the initial fractions with a monoclonal [an] antibody that is specific for caveolin and which binds to oligomerized caveolin in its native state as an oligomeric structural cage surrounding intact caveolae, and separating caveolae that are bound to the antibody from other materials in the initial fractions,

thereby producing purified caveolae.

24. (Amended) A method of producing purified caveolae, comprising the steps of:

a) providing a sample of plasma membranes from cells of interest;

b) subjecting the sample of plasma membranes to a membrane disruption method, thereby producing a disrupted plasma membrane sample;

c) subjecting the disrupted plasma membrane sample to a separation method based on density, thereby producing fractions of the disrupted plasma membrane sample, and collecting initial fractions of the disrupted plasma membrane sample;

d) subjecting the initial fractions of the disrupted plasma membrane sample to an immunoisolation method to separate caveolae from the initial fractions, wherein the immunoisolation method comprises incubating the initial fractions with a monoclonal [an] antibody that is specific for caveolin and which binds to oligomerized caveolin in its native state as an oligomeric structural cage surrounding intact caveolae, for a time period that is less than approximately 2 hours, and separating caveolae that are bound to the antibody from other materials in the initial fractions,

thereby producing purified caveolae.